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The aim of this project is to record the neural activity of serotonin (5HT) and dopamine (DA) neurons in rats during the performance of a reinforcement learning task. We are interested in how neural activity within these ascending modulatory systems is temporally related to events during learning and decision making. This work will provide a window onto the function of the DA and 5HT systems that is complementary to the rich to existing knowledge from pharmacological and biochemical assays. As there are no studies characterizing the behavior of 5HT neurons during the performance of a behavioral task in any species, a major goal of the work is to identify phasic behavioral correlates of 5HT neural activity. Over the last year, we have overcome many of the technical hurdles associated with recording from 5HT cells in awake behaving animals and in the process have made significant strides towards our goal of characterizing neural activity within the serotonin and dopamine systems during goal directed behavior.

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INTRODUCTION

The goal of this project is to record the neural activity of serotonin (5HT) and dopamine (DA) neurons in rats during the performance of a reinforcement learning task. We are interested in how neural activity within these ascending modulatory systems is temporally related to events during learning and decision making. Electrophysiological studies of ventral tegmental area (VTA) neurons in monkeys by Wolfram Schultz's group have led to a breakthrough in understanding of the DA system by showing that their phasic firing patterns correspond to an important variable posited by reinforcement learning theory, the reward error (or temporal difference) signal (1). These studies have not yet been replicated in rats. In doing so, the ongoing work will provide a window onto the function of the DA system that is complementary to the rich to existing knowledge from pharmacological and biochemical assays. Even less is known about the relationship between dorsal raphe 5HT neuron firing and decision variables in any species. Indeed, there are no studies characterizing the behavior of 5HT neurons during the performance of a behavioral task in any species. The major goal of the ongoing work is to identify phasic behavioral correlates of 5HT neural activity. Over the last year, we have overcome many of the technical hurdles associated with recording from 5HT cells in awake behaving animals and in the process have made significant strides towards our goal of characterizing neural activity within the serotonin and dopamine systems during goal directed behavior.

RESULTS

Anesthetized Experiments

Localizing and Identifying Single Cells within the Dorsal Raphe Nucleus

Forebrain projecting serotonin neurons are principally located within two midline nuclei – the dorsal raphe and median raphe nuclei. Because these nuclei consist of a mixture of cell types in addition to serotonin producing cells, it was important to establish our ability to reliably identify distinct cell classes within the raphe nucleus using standard electrophysiological criteria prior to the initiation of awake recordings. To this end, we carried out a series of experiments using sharp-tungsten electrodes in the anesthetized rat. Consistent with previous studies (2-3), we find at least two distinct cell types, examples of which are shown in Figure 1. The

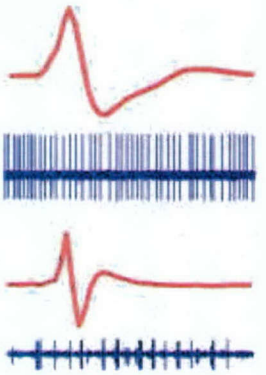


Figure 1: Example traces of neural activity within the raphe nucleus. *Top trace*: Serotonin neuron exhibits wide action potential and regular firing pattern *Bottom trace*: Gabaergic cell has narrow spikes and irregular firing

first cell class consists of putative GABAergic interneurons characterized by their narrow waveform and high firing rate. The second cell class consisting of cells with wide action potentials and spontaneous firing rates of around 0.5-2 Hz is characteristic of serotonin producing neurons. Ongoing pharmacological experiments are currently being used to insure that we have a reliable electrophysiological tag of serotonin cells capable of being applied to awake-behavioral recordings.

Transitioning from Tungsten Electrodes to Tetrodes: Technical Hurdles and Solutions

High impedance tungsten electrodes provide high signal to noise ratios with respect to the identification of single neurons. However, the impedance of these electrodes limits one's ability to record from more than one cell at a time. Although it is possible to record from more than one cell by reducing electrode impedance, there is a trade off in one's ability to discriminate between action potentials originating from different cells. Since we are especially interested in characterizing interactions between cells within the raphe, we decided to use tetrode (four channel (4)) recording electrodes rather than single tungsten wires for our behavioral experiments. By using a process analogous to triangulation, tetrodes are capable of providing reliable single cell isolation even in a low impedance regime thereby allowing for the simultaneous recording of several single cells within small brain regions such as the raphe nuclei. However, in the process of switching from tungsten electrodes to tetrodes a number of technical hurdles had to be overcome. In particular, the anatomical position of the raphe nuclei is such that the most direct electrode penetration passes thru both the midline sinus (plexus of blood vessels) and the cerebral aqueduct. While sharp

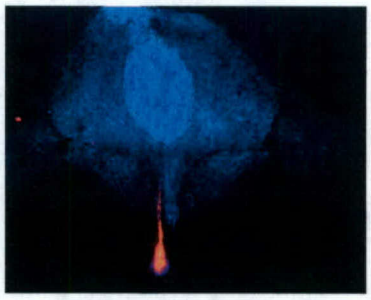


Figure 2: Targeting the raphe nucleus. Histological section showing a fluorescently labeled electrode within the raphe nucleus. Dark area is brain tissue, blue area is cerebral aqueduct, orange is the electrode tract.

electrodes are able to puncture the sinus and the thick dura below it, tetrodes are not stiff enough to pass through. While it is possible to bypass the sinus by implanting our electrodes at an angle relative to the midline, this approach limits our ability to record from both the dorsal and medial raphe nucleus on the same electrode penetration—a serious issue for long term chronic implants. As a consequence, we developed a novel two-step approach for tetrode targeting and implantation. In the first part of the procedure, a hole is drilled in the skull above the raphe (-7.8 mm A.P., 0.0 Lateral to bregma) and a stainless steel guide cannula (18.5 gauge) with a stylet to maintain patency is lowered into the brain.

The cannula tip is at a depth of ~ 4mm from the skull surface (~ 2mm above the raphe). The cannula thus creates a channel by which tetrodes can directly contact the brain. The animal is allowed to recover from the surgery after which the tetrode drive is implanted onto the cannula. Using this procedure we are able to stereotaxically localize tetrodes into the raphe nucleus as demonstrated by fluorescent labeling of tetrode tracks stained with DiI (Fig. 2). Thus, in the last year we have been able to effectively transition from tungsten to tetrode recordings in the raphe nucleus.

Awake Experiments

Monitoring and Controlling Behavior

A critical component of our proposal was to record from serotonin and dopamine containing neurons while animals performed a variety of goal directed behaviors. Over the last year, we have made significant progress in developing a battery of olfactory guided behavioral tasks (5). In particular, we have trained rats and mice in both a conventional go-no go task, and a novel two alternative olfactory discrimination task. The novel olfactory discrimination task provides a mechanism for parametrically varying both task difficulty (thru the use of odor mixtures) as well as various reward contingencies. Ongoing behavioral experiments are being use to quantify various behavioral parameters including reaction times and learning curves. The ability of both rats and mice to perform these complex olfactory discrimination tasks provides us with the ability to take advantage of the unique advantages of each species in understanding the role of neuromodulation during goal directed behavior. Specifically, the large size of rats in comparison to mice is much more amenable to simultaneous raphe and VTA recordings due to the fact that the relevant brain structures are larger in rats than mice, and rats can more easily carry larger electrode array. Mice on the other hand provide the opportunity to take advantage of powerful transgenic and knockout approaches in order to selectively disrupt spike transmission within 5HT and DA systems.

In addition to our behavioral developments, we have established a variety of analysis tools for data management as well as correlating physiological activity with behavioral performance (6). In particular, a key component of our proposal is to relate on going neuronal activity within the raphe and VTA to a particular motor pattern – namely the rodent breathing cycle. With respect to this goal, we have successfully monitored rodent sniffing patterns during behavior and have developed analytical tools for identifying and characterizing distinct sniffing patterns time locked to discrete behavioral events. As an outgrowth of this analysis, we have observed a novel step-like transitions in sniffing frequency time locked to the initiation of odor sampling and the anticipation of reward (Figure 3). These step like transitions suggest that rodent breathing patterns exist as discrete sets of sampling modes as opposed to a continuum of sampling frequencies (7) and establish a novel route of analysis with respect to subsequent neural recordings.

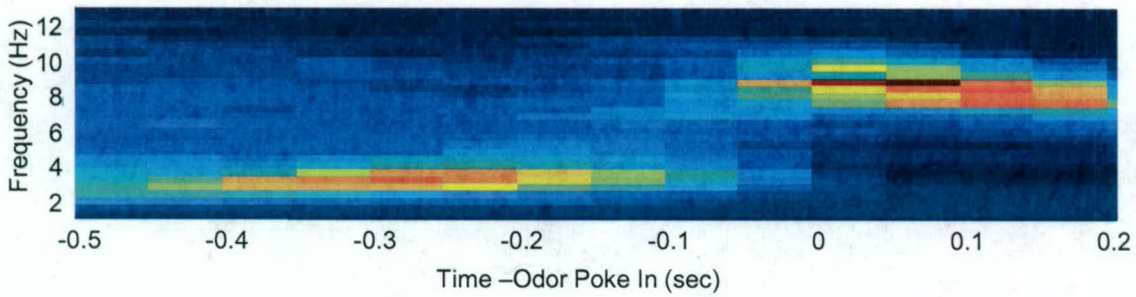


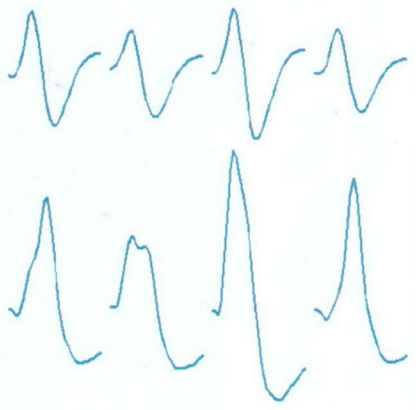
Figure 3: Step like transition in instantaneous sniffing frequency time locked to the intiation of odor sampling. Power spectrum: warm colors indicate strong power in a given frequency band, cool colors indicate low power. Sniffing frequency jumps from a 2-4 Hz mode to an 8-10 Hz mode just prior to the animal poking his nose into the odor sampling port

Raphe Recordings in the Awake Rat

In the last several months, we have successfully recorded neural activity from the raphe nucleus of awake animals under baseline conditions. A critical goal of these experiments was to determine the health and long term viability of subjects given the fact that the process of tetrode implantation involved potential damage to both the midline sinus and the cerebral aqueduct. In addition, because the tissue surrounding the raphe nucleus—periaqueductal gray (PAG)—is implicated in a number of defensive and aggressive behaviors we wanted to insure that our chronic implant did not cause undue behavioral disturbances. Observations of chronically implanted animals indicates that they are viable for period of time greater than that necessary to obtain the appropriate amount of electrophysiological data and that there are no gross behavioral deficits which may impair interpretation of the animals' performance on the relevant olfactory discrimination tasks.

Furthermore, electrophysiological data from these animals attest to our ability to obtain high quality electrophysiological recordings comparable to anesthetized conditions with respect to both signal to noise ratios and ability to discriminate different classes of neurons with the raphe nucleus (Fig. 4).

Figure 4: Example waveform averages for two cells recorded from the raphe nucleus of an awake rat. Each set of four traces corresponds to the four electrodes within a tetrode. *Top traces:* Narrow waveforms corresponding to a putative GABA containing interneuron. *Bottom traces*: Wide action potentials of a serotonin containing neuron within the DRN



KEY RESEARCH ACCOMPLISHMENTS

- Developed and characterized rodent behavior in a novel two-alternative olfactory discrimination task.
- Adapted rat behavioral paradigm to mice.
- Developed novel cannulated microdrive assembly for long term tetrode recordings in deep brainstem structures.
- Characterized the breathing patterns of rats during two-alternative olfactory discrimination task.
- Successfully recorded from the dorsal raphe nucleus in both anesthetized and awake rats.
- Developed electrophysiological criteria for identifying putative serotonergic and Gabaergic neurons within the raphe nucleus.

REPORTABLE OUTCOMES

1. Work toward the degree of Ph.D. for Sachin Ranade, Neurobiology and Behavior Program, Stony Brook University.
2. Obtained research support from the Thomas Hartman Foundation For Parkinson's Research.
3. Kepecs A, Uchida N, Maien ZF (2005) Rapid sniffing mode switch in anticipation of olfactory sampling. COSYNE 144.

CONCLUSIONS

Over the last year, we have made substantial progress in achieving the goals of our proposal by establishing the fundamental behavioral and electrophysiological techniques necessary for successful project completion. With respect to behavior, we have developed and characterized a novel two alternative olfactory discrimination task. By monitoring the respiratory patterns of rats as they perform this task, we have found stereotypical motor patterns during behavior – including a novel step-like transition in sniffing frequency locked to distinct behavioral events such as odor sampling and reward anticipation. With respect to electrophysiological accomplishments, we have overcome a number of technical hurdles in order to successfully record the neural activity of serotonin producing neurons within the raphe nucleus of both anesthetized and awake rats. Based on these recent technical accomplishments, we are now in the position of combining our electrophysiological and behavioral protocols in order to achieve the goal of linking neural activity within the raphe nucleus to cognitive and motor variables during the performance of a reinforcement learning task.

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